



THE MULTIDISCIPLINARY FIELD of tissue engineering (TE) brings together materials engineering, manufacturing engineering, and biology. One of the aims of TE is to deliver laboratory-grown tissues to either fully or partially replace organs and tissues that are diseased or malfunctioning due to genetic causes or aging, as well as those that were removed either due to surgical correction or trauma.

A foundational component of TE is the scaffold or construct, which is a substrate or platform to which cells are able to attach themselves and slowly grow into functional tissues [1]. Life sciences research has been transitioning from traditional 2D to 3D culture, since the cells behave very differently in 2D and 3D environments. A 3D microenvironment better represents the in situ native tissue microenvironment, and scaffolds offer such an in situ microenvironment ex situ [2]. Several considerations must be heeded in terms of scaffold design for TE, namely the material used and apparent mechanical properties of the scaffold and the architecture of the scaffold.

A minimum requirement for any material selected for use as a TE scaffold is that it is nontoxic. The material itself and the fabrication process of the scaffold should not induce toxic byproducts that can cause negative effects on cells. Therefore, biocompatibility, bioactivity, biodegradability, and nonimmunogenicity are the other factors that should be taken into consideration [3], [4]. Most classes of materials (ceramics, metals, and polymers) have been used as biomaterials for TE constructs. Of these, polymers are the most interesting as they hold great versatility. By modifying their chemical compositions, combinations, and molecular arrangements, biopolymers can be tailored to yield the properties and characteristics suited for tissue- and organ-specific TE applications [5].

The fabricated scaffold, whether it is a permanent implant or degradable, must

Specialized Multimaterial Print Heads for 3D Hydrogel Printing

Tissue-engineering applications.

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be able to hold its form upon the initial implantation. The set of mechanical properties that achieves this should be modulated to be consistent with the surrounding tissues and not be overcompensated, as mismatch may cause adverse consequences. A classic example is the case of metallic implants that have elastic stiffness that is inherently much higher than bone, which causes a phenomenon known as *stress shielding*, in which bone reabsorbs as the lack of imposed stresses signals sufficient bone of a particular strength [6].

Scaffolds, especially those used for 3D translational applications such as bone TE, are required to have a specific microarchitecture that allows nutrients to flow deep into the engineered tissue and remove metabolic waste to avoid toxic buildup. Pores of the scaffolds should be close enough to allow proper cell attachment and also wide enough to not impede cell migration and infiltration. On a similar note, the supporting infiltration of native vasculature maintains long-term survival of the engineered tissue [7]; thus, the architecture should also allow and facilitate vascularization. Pore size dictates the transfer of oxygen and carbon dioxide, which is also vital for proper tissue function and cell survival. The given architectural and property requirements are summarized by the umbrella term *biomimicry*, which essentially is when a scaffold used for TE is expected to mimic the native tissue in architecture, mechanical strength, and stiffness, and also possess other physical, biochemical, and biological attributes [8].

ADDITIVE MANUFACTURING OF TE SCAFFOLDS

While several methods fabricate 3D porous scaffolds, such as freeze-drying, gas foaming, solvent casting, and electrospinning [9], constructing a biomimetic architecture with these methods is challenging, given the inherent limitations and stochastic nature of these approaches. Additive manufacturing (AM), commonly referred to as *3D printing* (3DP), is a versatile digitally controlled fabrication technology capable of the one-stage production of intricate volumetric shapes. Use of the technology began in the 1980s and was patented in 1986 as stereolithography apparatus (SLA). Objects are built in a layer-by-layer fashion

using materials that can take form rapidly enough, before the succeeding layer is laid above, to yield a 3D structure.

The use of AM has many advantages compared to the traditional scaffold-fabrication methods. In addition to the geometric freedom and potential for biomimetic designs since the design is CAD, tuning the scaffold architecture is easier and more flexible. The accuracy and repeatability of the AM processes would produce consistent scaffold quality. In other words, AM has the ability to fabricate biomimetic scaffolds, representing complex, internal tissue microarchitecture. For example, emerging scaffold designs such as triply-periodic minimal surface architecture [8] that mimic bone structure and functionally gradient scaffolds [1] that are otherwise challenging to fabricate can be now realized with the advent of AM.

3DP technology has found its way into TE from the beginning of this revolutionary medical intervention, as it provides the best approach to develop scaffolds. In 3DP, the structure to be printed is first conceptualized digitally using 3D modeling software (AutoCAD, SolidWorks, etc.). The models to be created are then converted into a stereolithography file (.STL), which is a tessellated version of the original file. This file is later sliced, generating a G-code (which contains all of the steps for the 3D printer to follow) that will be sent to the 3D printers for fabrication. In the medical field, .STL files can also be obtained from the DICOM images acquired from computerized tomography/magnetic resonance imaging, facilitating the production of patient-specific joint-replacement implants.

It is interesting to note that the computational methods are used not only for the design or modeling of scaffolds, but also to more deeply analyze the scaffold design to establish the structure-property relationships such as porosity versus stiffness or pore size versus strength, the simulation of fluidic properties such as permeability and oxygen diffusion, predicting the degradation behavior of scaffolds, and the topology optimization of the scaffold structures [10]–[12]. Using computational methods for TE scaffolds is comprehensively summarized in a recent review [13].

Several types of AM processes are grouped under seven categories by the American Society for Testing and Materials [14]. They are vat photopolymerization, material jetting, binder jetting, material extrusion, powder bed fusion, sheet lamination, and directed energy deposition. The basic working principles, examples, and the pros and cons of each of these seven categories are summarized in Table 1. Only some of the AM processes that fall under these seven categories could be used to fabricate 3D TE scaffolds. A few of them are preferred due to constraints such as limited material requirements, resolution, accuracy, ease of fabrication, and cost. The three most commonly used AM processes for TE scaffold fabrication are SLA, selective laser sintering (SLS), and fused deposition modeling (FDM). We will briefly introduce each of these techniques and present limited studies to highlight each technique.

SLA

SLA is a high-resolution process and one of the earliest AM methods used for TE scaffold fabrication. It involves light-mediated curing, or the polymerization of photopolymer resin kept in a bath. The light source is usually ultraviolet (UV) and when the photocurable resin is exposed to UV light, polymerization takes place and the layer is solidified. The process is repeated layer by layer to form the 3D object. Its main limitation is that only photocurable polymers, such as polyethylene glycol acrylate and polypropylene fumarate (PPF) or resins with photoinitiators, can be used.

The SLA method has been explored for fabricating bone TE scaffolds using a biodegradable resin mixture of PPF, diethyl fumarate, and bisacrylphosphine oxide [9]. In another study, photocurable poly(ϵ -caprolactone) (PCL)-based resin was 3D printed into TE scaffolds with a porosity of 70% and a pore size of 465 μm [15]. Demonstrating further versatility using polymers and AM, boron nitride nanoplatelets containing photopolymer were successfully fabricated using SLA into TE scaffolds with increased compressive strength [16]. While there are many such studies, only a few are given as examples here and for the methods described.

TABLE 1 The categories of the additive manufacturing processes.

CATEGORY	WORKING PRINCIPLE	EXAMPLES	PROS	CONS
Vat photopolymerization	A 3D structure is created by selectively curing a bath of photopolymer resin using either UV or visible light.	SLA	• High accuracy • Scalability • Relatively faster	• Expensive • Limited material range • Lengthy postprocessing
Material jetting	Material in the form of droplets is jetted through a nozzle, either continuously or on demand.	Inkjet printing, continuous or drop on demand	• High accuracy • Multimaterial printing	• Support material • Limited material range
Binder jetting	A 3D structure is created by selectively binding the build material (usually in powder form) using liquid binding agents.	Binder Jetting	• Large range of materials (metals, ceramics, and polymers) • Relatively faster	• Parts lack structural strength • Lengthy postprocessing
Material extrusion	Material in the form of either a liquid or filament is extruded through a nozzle to form a 3D object.	FDM	• Low cost • Ease of use	• Low accuracy • Low resolution
Powder bed fusion	A 3D structure is created by selectively melting and fusing powder material in a bed using either a laser or electron beam.	Direct metal laser sintering, selective laser melting, and selective laser sintering	• No support structures • A large range of materials	Powder material quality (grain size, etc.) influences the final product quality
Sheet lamination	Sheets of materials are bonded together to form 3D objects.	Laminated object manufacturing and ultrasonic AM	• Low cost • Ease of use	Limited material range
Directed energy deposition	Material (either powder or wire) is fed through a nozzle and is melted using a laser and deposited to form 3D objects.	Laser-engineered net shaping, 3D laser cladding	• High quality • Relatively faster	Limited material range

SLS

SLS creates solid structures by tracing and focusing a laser onto a thin layer of the powder of choice. The laser melts and fuses the powdered material together to form a solid connected pattern [17]. SLS's main limitation is the requirement that the raw material has to be in powdered form and that the material must be able to withstand heat from the laser, resistant to high temperatures, and less prone to shrinkage [18]. SLS is particularly preferred when scaffolds with high mechanical strength are required, for example bone TE applications. SLS successfully processed scaffolds with different material combinations, such as polyvinyl alcohol/hydroxyapatite (HA) [19], poly(l-lactide)/carbonated HA [20], calcium phosphate/poly(hydroxybutyrate-cohydroxyvalerate), and carbonated HA/poly(l-lactic acid) [21].

FDM

FDM is one of the most widely used techniques because it does not require lasers and is inexpensive [3]. It requires low melting point polymer filaments that may be biodegradable and biocompatible. The two most commonly used thermoplastics in FDM are polylactic acid and acrylonitrile butadiene styrene (ABS). Like

SLA and SLS, FDM's main limitation is the choice of raw material used; it must be thermoplastic and most commercially available filaments, such as ABS, polycarbonate, or polyphenylsulfone, are not suitable for scaffold printing since they are not biocompatible. PCL and PCL-based filaments are extensively explored in the literature for TE scaffold printing using FDM [22]. Some examples include PCL-tricalcium phosphate scaffolds [23]–[25] and PCL/HA scaffolds [26] for bone TE. Several detailed reviews on scaffold printing using these techniques are available elsewhere [27]–[29].

BIOPRINTING

The scaffolds fabricated by 3DP for TE applications can be classified as either acellular or cell laden. Acellular scaffolds do not contain cells but possess biological components that purposefully engage in cell recruitment, enhance cell attachment, direct cell differentiation, and encourage cell proliferation once implanted. These activities allow patient cells to populate the scaffolds to regenerate tissues after implantation. These acellular scaffolds come under the umbrella domain of AM/3D printing while cell-laden scaffolds, or rather cell embedded

within tissue constructs, would come under a subset of AM called *bioprinting*.

While most of the acellular scaffolds discussed focus on hard tissues, such as bone (there are exceptions), it can be loosely claimed that bioprinting focuses on hydrogel-based cell-laden scaffolds for soft tissues. Hydrogels are biopolymers that can be formulated from naturally derived and synthetic material [30]. They are soft and tissue-like and have the ability to contain 70–90% water. As a scaffold, they present cells with a biomimetic microenvironment [30], [31]. When cells come into play, the requirements of the process change drastically. For example, SLS, which operates at a high temperature, cannot be used to fabricate cell-laden scaffolds and FDM, which is filament based, cannot be used also because cells have to be suspended in liquid-based media. However, bioprinting has different specifications that cater to a unique cell-friendly environment even though it is a subset of 3DP.

Bioprinting processes can be broadly grouped under four categories: laser based, droplet based, extrusion based, and stereolithography bioprinting [32]. The detailed classification is summarized in Figure 1 and the schematics of each of the processes are shown in Figure 2.

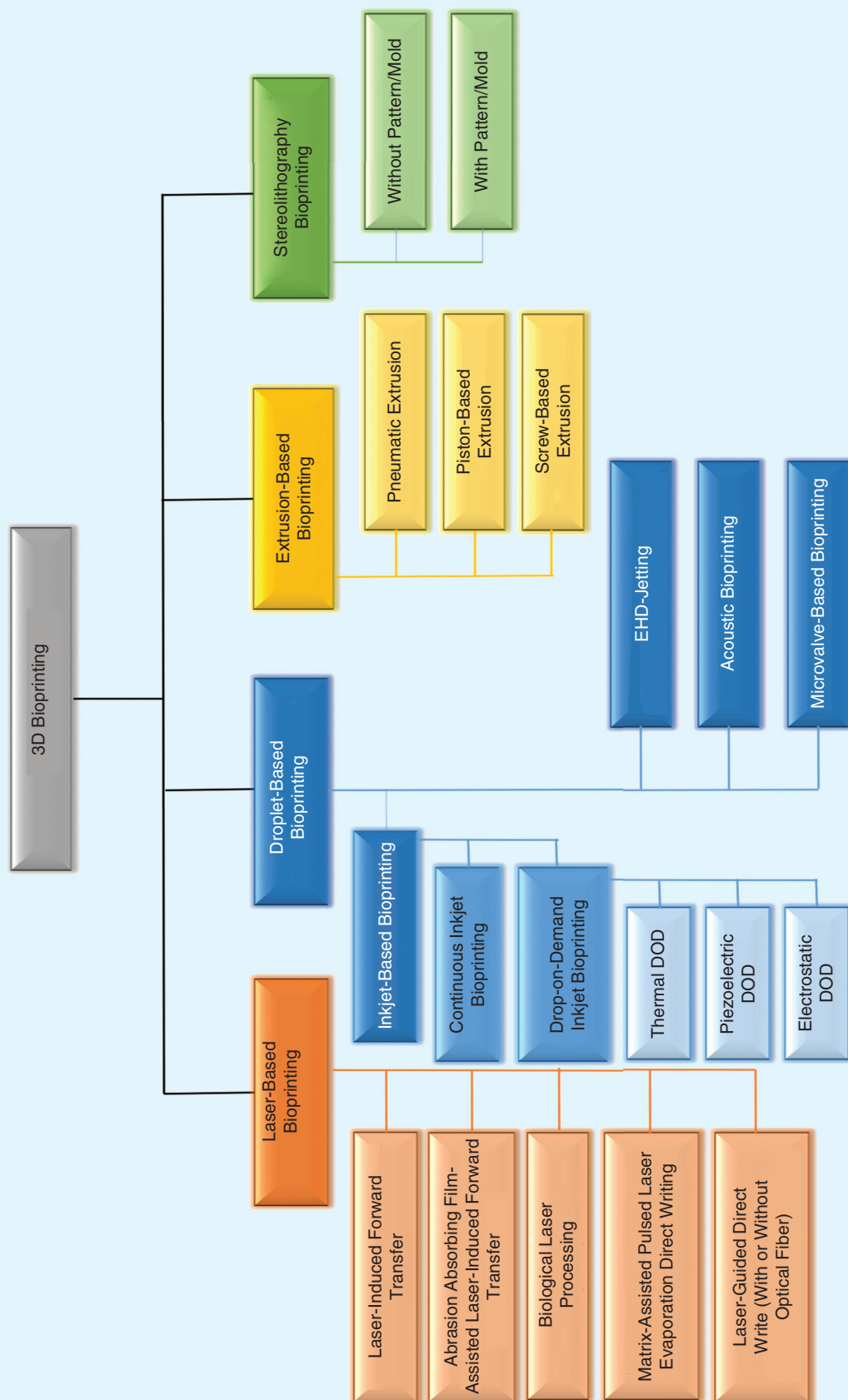


FIGURE 1 The classification of bioprinting processes. Among the four 3D bioprinting processes, extrusion-based methods are used the most, considering their cell-friendly printing approach. Most of the μ fabricated print heads are attached to extrusion-based bioprinters just at the outlet of the print. EHD: electrohydrodynamic; DOD: drop-on-demand. (Used with permission from [39].)

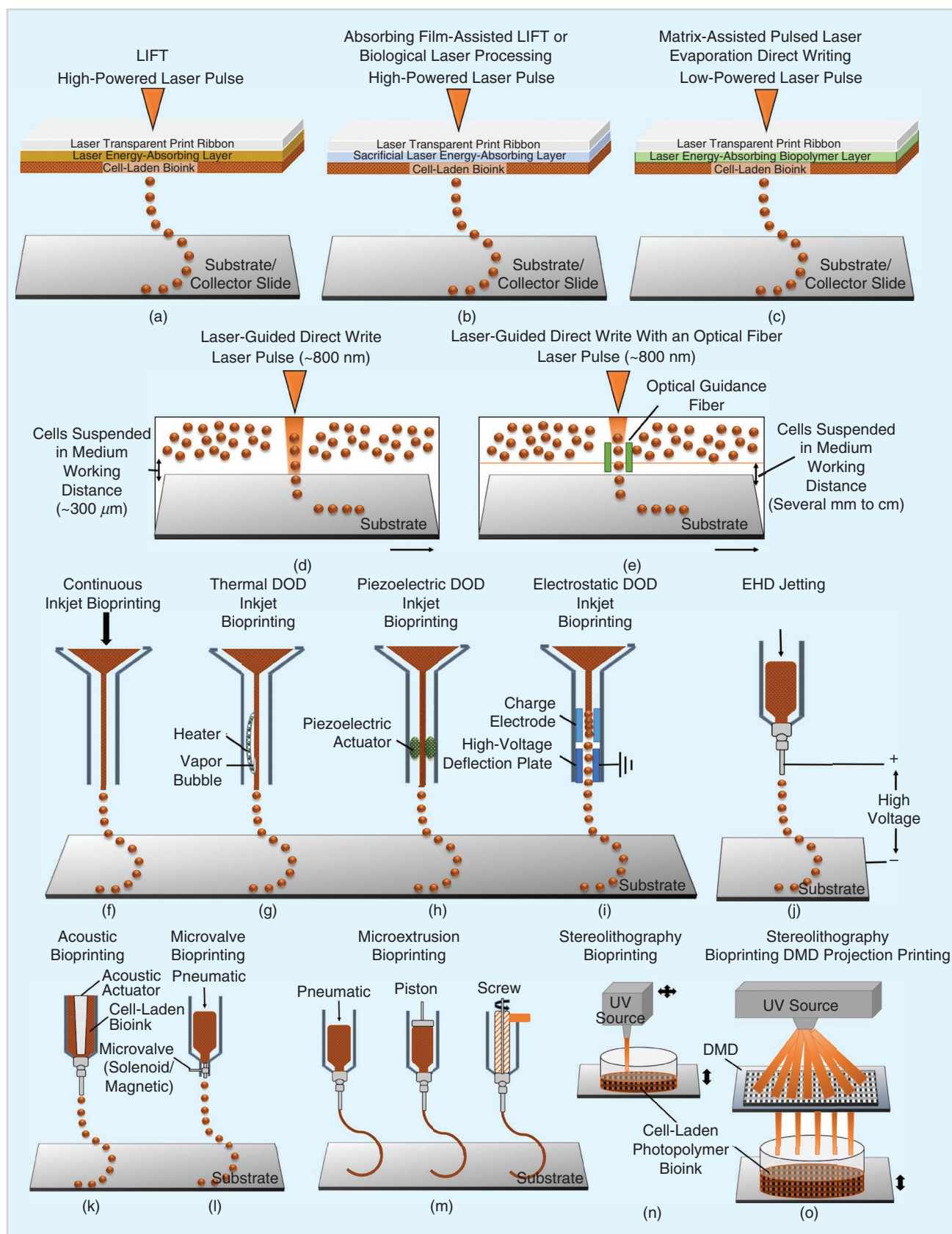


FIGURE 2 The 3D bioprinting processes. (a)–(o) The key working principles of each of the bioprinting processes. DMD: digital micromirror device; UV: ultraviolet. (Used with permission from [39].)

A brief introduction to each of the above processes is given here, as detailed reviews on these processes are available elsewhere [32]–[35].

LASER-BASED BIOPRINTING

In laser-based bioprinting, laser energy propels droplets of cell-laden hydrogel onto the substrate. Laser-induced forward transfer (LIFT) [Figure 2(a)] is the most common process, although there are other laser-based processes depending on the type of laser source and the print layer, such as absorbing film-assisted LIFT [Figure 2(b)], biological laser processing [Figure 2(b)], matrix-assisted pulsed laser evaporation direct writing [Figure 2(c)], and laser-guided direct writing [Figure 2(d) and (e)]. The many advantages of laser-based bioprinting include high postprinting cell viability, as it is a noncontact process (unlike nozzle-based) and, hence, there is less chance of contamination, high resolution, and the ability to print high cell densities. However, the laser's effect on cells, such as photonic cell damage [36], prevents the wide use of this process.

DROPLET-BASED BIOPRINTING

Droplets of cell-laden hydrogels are deposited onto the substrate in droplet-based bioprinting. Based on the source of energy or working principle, there are several processes under droplet-based bioprinting, which include inkjet bioprinting, EHD-jet bioprinting, acoustic bioprinting, and microvalve bioprinting (Figure 2).

INKJET BIOPRINTING

Inkjet printing was originally developed for graphics and text printing to form eligible patterns with the presence or absence of the ink. The drops are first directed and applied to a specific location on the target and there will be interactions between the drop and target. Unless using hydrogels as the bioink, applications using inkjet printing are mainly 2D, with the predominant application being patterning of biomolecules and cells [37]. Inkjet printing can be further classified into continuous inkjet (Figure 2f) and drop-on-demand inkjet printing [Figure 2(g), (h), and (i)].

EHD-JET BIOPRINTING

EHD-jet printing [Figure 2(j)] is a high-resolution process that utilizes a strong electric field force (a high voltage applied between the nozzle and the substrate) to attract the cell-laden hydrogel droplets onto the substrate continuously [38]–[41]. The high-resolution (down to 100 nm) and high-viscosity bioink printing abilities are advantageous, while the inability to generate on-demand droplets of bioink for spatial arrangement and the viability of cells exposed to high voltage are disadvantages.

ACOUSTIC AND MICROVALVE BASED BIOPRINTING

Acoustic bioprinting [Figure 2(k)] is a relatively new process that uses acoustic waves to generate droplets of bioink on demand [42]. A high resolution (37 μm) and a rapid printing speed are advantages of this process, while stress associated with the high-viscosity bioinks processed using this technique is detrimental to the cells. Microvalve bioprinting [Figure 2(l)] uses solenoid or electromechanical valves to eject droplets of bioinks onto the substrate [43]. Less pneumatic pressure compared to inkjet printing and synchronized multihead printing are the greatest advantages of microvalve bioprinting, but low printing speed, low resolution, and clogged nozzles are its limitations.

EXTRUSION BIOPRINTING

Of all of the bioprinting processes, extrusion bioprinting is the most commonly used [Figure 2(m)]. Mechanical force (piston or screw-based) or pneumatic pressure is used to extrude the bioink out of the nozzle. If there is one bioprinting method that has the potential to fabricate scalable

tissues, it is extrusion bioprinting; it offers the greatest advantage of this process. The capability to print high-viscosity bioinks and high cell concentration are other advantages. The limitations of this process include low resolution and clogged nozzles.

STEREOLITHOGRAPHY BIOPRINTING

The working principle of stereolithography bioprinting [Figure 2(n) and 2(o)] is similar to the SLA process described before, but the photopolymer and the photoinitiator used in the process must be nontoxic because cells are suspended in it and the light used is less intense. High resolution and high printing speeds are advantages of this process. However, nontoxic and biocompatible photopolymers are scarce, which limits it from being used widely.

PARAMETERS AFFECTING BIOPRINTING RESOLUTION

The resolution or minimum feature size requirements vary with tissues. The range of resolution required varies from $<5 \mu\text{m}$ (neurites, blood capillaries) to $500 \mu\text{m}$ (bone tissue) [44], so the choice of bioprinting approach should be based on the tissue type. Some tissues that have a functionally gradient architecture or zonal arrangement, such as cartilage or skin, would require a large range of feature sizes while a hybrid bioprinting approach might be required. Several process parameters affect the resolution of the bioprinted construct. Most common bioprinting parameters are bioink viscosity, cell density, printing speed, and nozzle type. A comparison of resolution and the range of process parameters for the four bioprinting types is shown in Table 2.

TABLE 2

The resolution and range of process parameters for various bioprinting approaches. mPa.s: millipascal-second. (Source: [44]–[46].)

PARAMETERS	LASER BASED	DROPLET BASED	EXTRUSION BASED	SLA BASED
Resolution	10–100 μm	10–50 μm *EHD: 3–10 μm	$>100 \mu\text{m}$	$>100 \mu\text{m}$
Bioink viscosity	1–300 mPa.s	$<10 \text{ mPa.s}$ *EHD: 1–100 Pa.s	10^6 – 10^7 mPa.s	1–300 mPa.s
Cell density	10^7 – 10^8 cells/mL	$<10^6 \text{ cells/mL}$	$\sim 10^9 \text{ cells/mL}$	$<10^8 \text{ cells/mL}$
Printing speed	200–1,600 mm/s	$\sim 100,000 \text{ droplets/s}$	700 mm/s– 10 $\mu\text{m/s}$	200–1,600 mm/s
The asterisk refers to the resolution of the specific droplet-based method EHD-Jet Bioprinting.				

In addition to the individual effects of these parameters on the resolution, the interplay between them also must be considered. For example, the higher the cell density, the higher the viscosity of the bioink will be; the viscosity of the bioinks should be considered together with the cell density rather than considering them as separate parameters. Similarly, higher viscosity bioinks might limit the printing speed, where higher printing speeds would result in discontinuous printing.

SPECIALIZED PRINT HEADS

The bioprinting methods mentioned mostly rely on the use of needles or pipette tips, except for the laser-based methods, which release droplets from a hydrogel substrate, or UV-based methods, which polymerize specific regions of a photopolymer. In the case of methods using needles, the needle will be modified depending on the specific technique. For instance, adding heaters, piezoelectric actuators, or electrodes. However, the limitation that only a single type of bioink can be used from a single print head or nozzle remains.

Using only one type of bioink implies a single cell type or that a uniform mixture of cells within the same bioink will be printed, which is limiting [47], [48]. In vivo, clear spatial heterogeneity within tissue compartments of the anatomy exists. The basic differences of each compartment could manifest in the form of 3D spatial geometry, tissue material, and cell type. For example, there is compartmental distinction between the tunica intima, tunica media, and tunica externa for blood vessels. The tunica intima are on the endothelial cells in the form of a 2D endothelial sheet, smooth muscle cells are within the 3D tunica media, and finally, there are no cells in the tunica externa.

The gold standard for bioprinting replacement organs would be to print the various tissue compartments with geometrical accuracy, with the appropriate tissue properties and cell types. Because cells are sensitive to their microenvironment, based on the biochemical and biomechanical cues presented to them, they will physiologically react and function differently depending on the type

of hydrogels used. This phenomenon is based on a field known as *mechanobiology*; it is reviewed elsewhere [49], [50]. Even when using the same hydrogel type, just based on differential hydrogel concentrations used, cells behave differently. In our laboratory, we have shown smooth muscle cells changing phenotype when cultured on collagen hydrogels with concentration changes [51].

Current bioprinting practices can allow such heterogeneity through changing the bioink, sequential printing, or multiple print heads/nozzles that each have a separate bioink. These setups usually result in prints that possess relative poorer resolutions, low physical and chemical integration at the bioink interfaces, weakened mechanical stability during the print due to excessive stage movement, and the inability to have gradient material properties [47]. In addition, the printing time is prolonged [34], [52], which could lead to contamination of the cell culture system, premature drying of hydrogels, and low cell viability. Printing duration can also be further extended due to priming of the dispensing system or preventing bubble formation within the dispensing system [47]. It is worthwhile to mention that Lee et al. developed a carousel-like system, holding reservoirs of different bioinks to alleviate the issues of prolonged printing time [53].

Several solutions for continuous printing that dispense multitype bioinks from a single source have been reported and the remainder of this review will be dedicated to showcasing these technologies. In most of the examples given, these multiple material print heads are custom microfabricated (μ -fabricated) and attached at the endpoint of extrusion-based bioprinters just before print. By custom μ -fabricating print heads, designs can be incorporated to facilitate the printing of certain hydrogels, provide a milder fabrication environment for cell protection, and improve the resolution and precision [54].

This multinozzle print head consists of commercially available blunt needles and metallic tubes assembled together, in a telescopic configuration, to provide a solution for freeform 3D

printing [Figure 3(a)] [55]. Using alginate hydrogel doped with different dyes, Rocca et al. demonstrated bioprinting of structures of complex patterns containing multiple materials [55]. While the print head is not μ -fabricated per se, the assembled print head alleviates limitation in freeform 3DP. This exciting form of 3DP does not require the painful and tedious removal of supporting material, as this support material is often needed to facilitate the 3D formation of mechanically inept hydrogels. Freeform printing dispenses fast polymerizing hydrogels that solidify to a gelatin slurry that can be removed by simply increasing the ambient temperature to that of the body [56].

Scaling up, Liu et al. bundled seven capillary tubes into a single print head to pneumatically drive the extrusion of several types of carefully controlled gelatin hydrogels into intricate printed forms [Figure 4(a)] [57]. The hydrogels are composites, containing suspensions of nanosilicates that give them unique shear-thinning properties and they are extruded by a dedicated pneumatic drive.

When these custom or μ -fabricated micronozzles are arranged coaxially, solid or hollow hydrogels of varying configurations of hydrogels and cell content can be extruded [Figure 3(b)] [57]. This process has been successful in developing tubular tissue constructs with potential applications in vessel TE or kidney tubules from gelatin- and alginate-based hydrogels [58]–[60]. These coaxial nozzle designs can be extrapolated to provide extrusion of multiple bioinks in tubular form [Figure 4(b)]. While coaxially extruded hydrogel tubes are predominantly applied for tubular anatomical features, they have also been used for bulk tissue regeneration via direct writing. A microfluidics-based direct writer by Ghorbanian et al. guides tubular hydrogels based on digital information and lays them in 3D spatial space, achieving structures that are architecturally similar to scaffolds printed from stiffer biomaterials [61].

While results from bundling micronozzles have been remarkable, the resolution of the prints depends highly on the

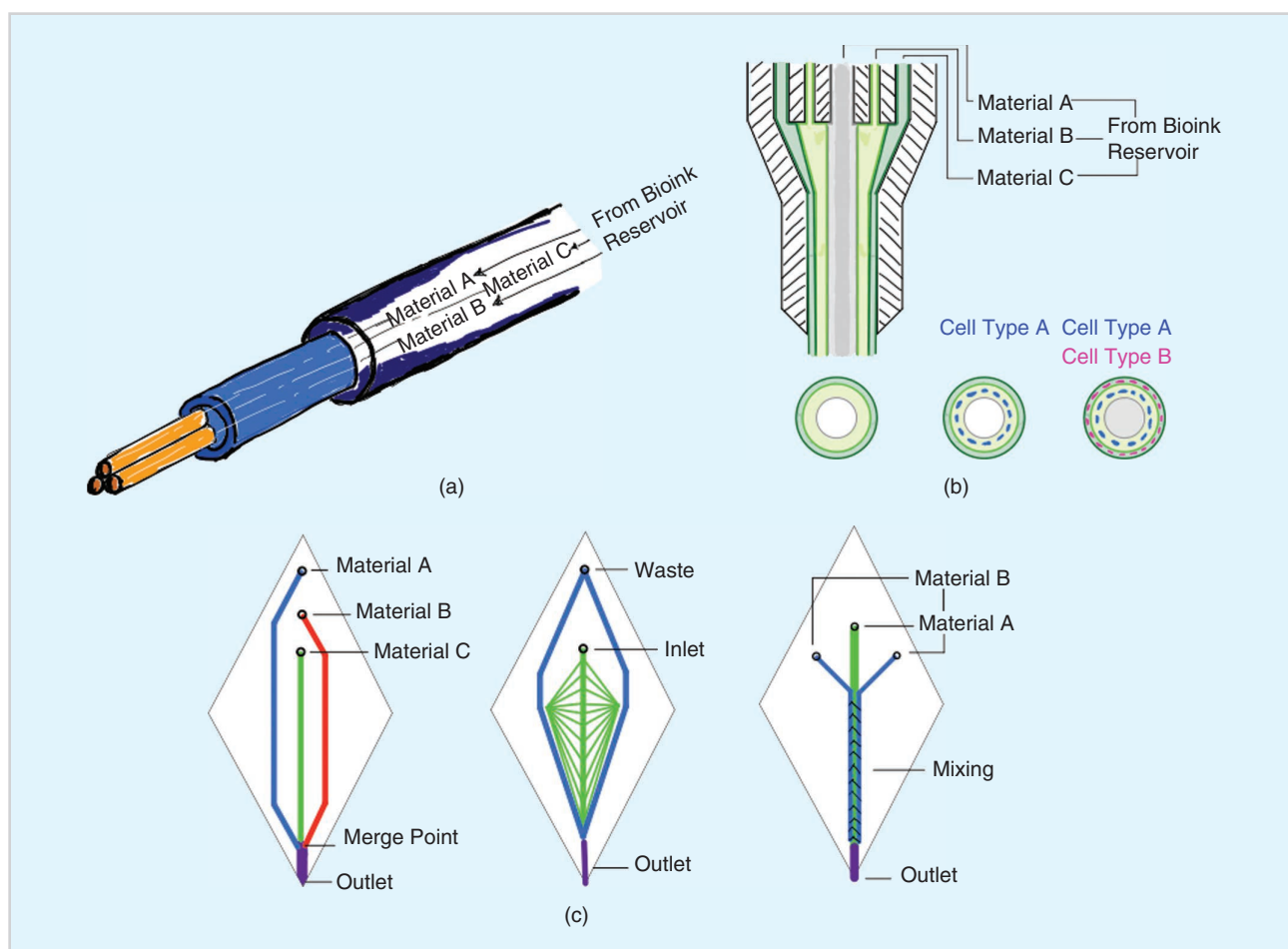


FIGURE 3 The μ -fabricated print heads for bioprinting multimaterial hydrogels. Custom print heads for 3D bioprinting of hydrogels are μ -fabricated and assembled as three general forms. (a) The micronozzles are bundled together to form an extrusion nozzle. This allows for different materials to be extruded simultaneously or sequentially via carefully controlled and timed processes to avoid intermixing the different materials prior to extrusion. Up to seven micronozzles have been attempted. (Used with permission from [57].) (b) The coaxial nozzles can extrude hydrogels scaffolds of different configurations of material and cell composition. (Used with permission from [58].) (c) The custom print heads can produce hydrogels at fine resolutions, using the same fabrication techniques to realize microelectromechanical systems. These can also be designed to precisely control the number of cells and their dispersion within cell-laden hydrogels. Finally, proper mixing of multiple types of hydrogels can also be achieved by using a mixing chamber design [62]. The schematics were drawn for illustrative purposes and as an adaptation.

diameters of the nozzles and how physically tight they can be bundled together. Serex et al. separately integrated different μ -fabricated microfluidic systems at the tip of the print head of an extrusion-based 3DP for alginate hydrogels [Figure 3(c)] [62]. Individually, these systems demonstrated fast switching between two or more materials for multimaterial printing that can be used to switch between different bioinks, with or without cells. They also showed the possibility of enhancing resolution of hydrogel printing using sheath flow [Figure 4(c)]. Finally, incorporating the micromixers at the print head makes it possible to insert cells into the bioink or create a gradient of compositions of cells into the final print.

FUTURE PERSPECTIVE

The reviewed approaches demonstrate the possibility of multimaterial, or multicompartamental, printing of tissues and organs. Multimaterial bioprinting, cell-laden or otherwise, is currently limited in terms of choice of bioinks. Fast polymerizing naturally derived bioinks or their hybrids are choice hydrogels, transitioning from a liquid precursor to its solid form in a timescale of milliseconds. While many studies have shown TE application of these hydrogels, the real challenge will be to use collagen hydrogel where complete polymerization is in the order of minutes. Collagen hydrogels in their pure forms are the predominant protein in mammals, as compared

to fast polymerizing alginates, gelatins, and fibrins. Currently, the use of collagen hydrogel stands at 3DP systems being employed to accurately deposit the cell-laden scaffolds into microfluidic devices to accurately recapitulate organ systems on chip [54], [63]. Going forward, it makes good sense to devote resources to facilitate 3DP of multimaterial and multicompartamental collagen-based scaffolds for soft tissue TE applications.

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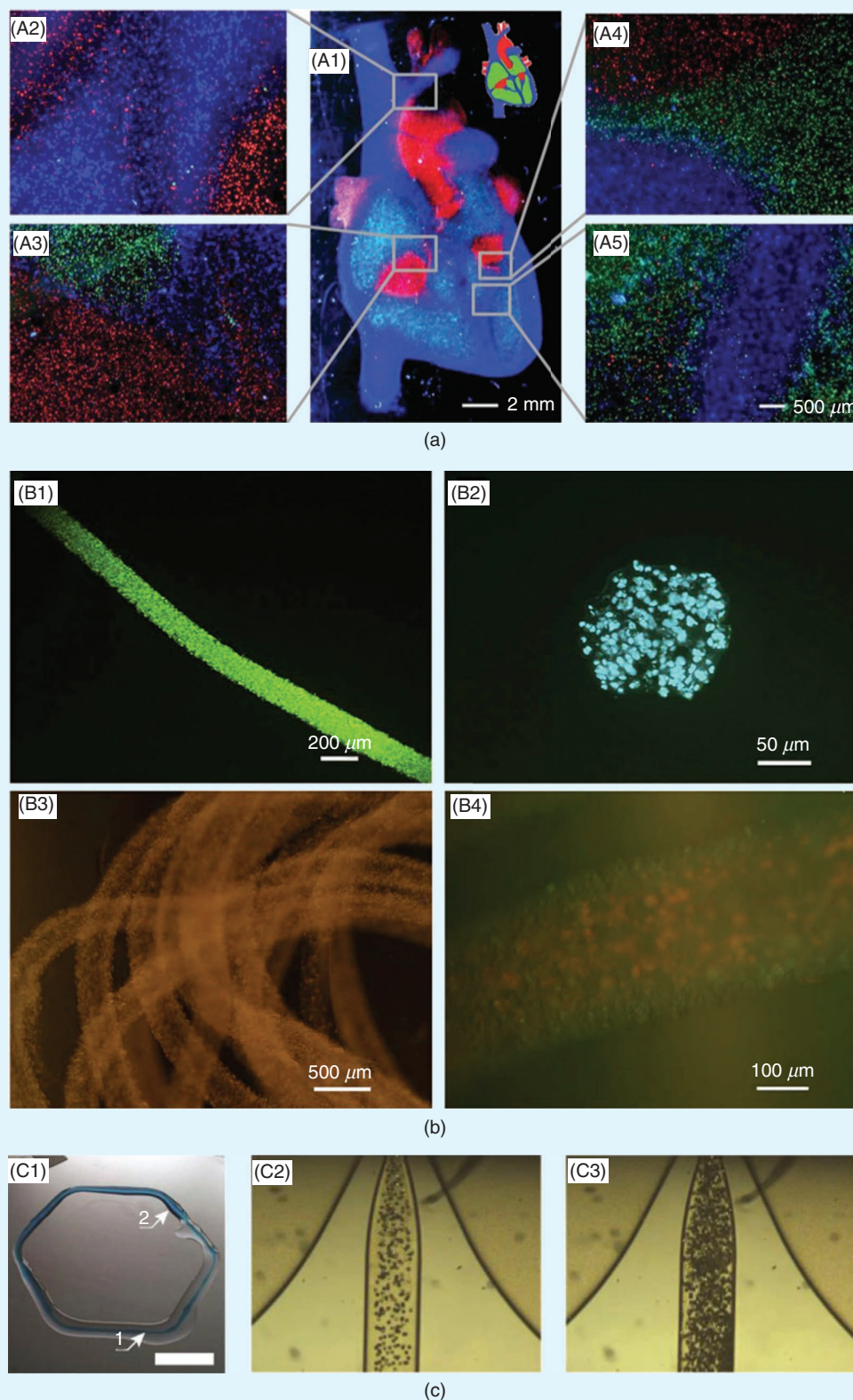


FIGURE 4 The tissue-engineering examples from bioprinting multimaterial hydrogels from μ -Fabricated print heads. (A1) Heterogeneous tissue printing by the extrusion nozzle by Liu et al. [57] was demonstrated through printing cell-laden hydrogels into distinct regions, representing a heart-like structure. (A2–A5) The cells within each hydrogel were marked with varying fluorescence to show distinctive regions. For TE of tubular anatomical features, the coaxial nozzle by Hu et al. [58] showed varying capabilities. (B1 and B2) The extruding cell-laden homogenous solid fibers and (B3 and B4) cell-laden hollow fibers with distinct regions. (C1–C3) Finally, the nozzle by Serex et al. [62] was able to establish cell-laden high-resolution hydrogel prints, achieved via hydrodynamic focusing. (Used with permission from [57], [58], [62].)

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REFERENCES

- [1] S. Vijayavenkataraman, S. Zhang, W. F. Lu, and J. Y. H. Fuh, "Electrohydrodynamic-jetting (EHD-jet) 3D-printed functionally graded scaffolds for tissue engineering applications," *J. Mater. Res.*, vol. 33, no. 14, pp. 1999–2011, 2018. doi: 10.1557/jmr.2018.159.
- [2] K. Duval et al., "Modeling physiological events in 2D vs. 3D cell culture," *Physiology (Bethesda)*, vol. 32, no. 4, pp. 266–277, 2017. doi: 10.1152/physiol.00036.2016.
- [3] A. Gleadow, D. Visscher, J. Yang, D. Thomas, and J. Segal, "Review of additive manufactured tissue engineering scaffolds: Relationship between geometry and performance," *Burns Trauma*, vol. 6, p. 19, Dec. 2018. doi: 10.1186/s41038-018-0121-4.
- [4] F. J. O'Brien, "Biomaterials & scaffolds for tissue engineering," *Mater. Today*, vol. 14, no. 3, pp. 88–95, Mar. 2011. doi: 10.1016/S1369-7021(11)70058-X.
- [5] D. F. Williams, "Challenges with the development of biomaterials for sustainable tissue engineering," *Front. Bioeng. Biotechnol.*, vol. 7, p. 127, 2019. doi: 10.3389/fbioe.2019.00127.
- [6] D. Apostu, O. Lucaciu, C. Berce, D. Lucaciu, and D. Cosma, "Current methods of preventing aseptic loosening and improving osseointegration of titanium implants in cementless total hip arthroplasty: A review," *J. Int. Med. Res.*, vol. 46, no. 6, pp. 2104–2119, June 2018. doi: 10.1177/0300060517732697.
- [7] S. Saberianpour, M. Heidarzadeh, M. H. Geranmayeh, H. Hosseinkhani, R. Rahbarghazi, and M. Nouri, "Tissue engineering strategies for the induction of angiogenesis using biomaterials," *J. Biol. Eng.*, vol. 12, p. 36, 2018. doi: 10.1186/s13036-018-0133-4.
- [8] S. Vijayavenkataraman, L. Zhang, S. Zhang, J. Y. Hsi Fuh, and W. F. Lu, "Triply periodic minimal surfaces sheet scaffolds for tissue engineering applications: An optimization approach toward biomimetic scaffold design," *ACS Appl. Bio Mater.*, vol. 1, no. 2, pp. 259–269, 2018. doi: 10.1021/acsabm.8b00052.
- [9] A. G. Mikos and J. S. Temenoff, "Formation of highly porous biodegradable scaffolds for tissue engineering," *Electron. J. Biotechnol.*, vol. 3, no. 2, pp. 114–119, 2000. doi: 10.2225/vol3-issue2-fulltext-5.
- [10] J. C. M. Teo and S. H. Teoh, "Permeability study of vertebral cancellous bone using micro-computational fluid dynamics," *Comput. Meth. Biomech. Biomed. Eng.*, vol. 15, no. 4, pp. 417–423, Jan. 2012. doi: 10.1080/10255842.2010.539563.
- [11] J. C. M. Teo, K. M. Si-Hoe, J. E. L. Keh, and S. H. Teoh, "Relationship between CT intensity, micro-architecture and mechanical properties of porcine vertebral cancellous bone," *Clin. Biomech.*, vol. 21, no. 3, pp. 235–244, 2006. doi: 10.1016/j.clinbiomech.2005.11.001.
- [12] J. C. M. Teo, K. M. Si-Hoe, J. E. L. Keh, and S. H. Teoh, "Correlation of cancellous bone micro-architectural parameters from microCT to CT number and bone mechanical properties," *Mater. Sci. Eng. C*, vol. 27, no. 2, pp. 333–339, 2007. doi: 10.1016/j.msec.2006.05.003.
- [13] S. Zhang, S. Vijayavenkataraman, W. F. Lu, and J. Y. H. Fuh, "A review on the use of computational methods to characterize, design, and optimize tissue engineering scaffolds, with a potential in 3D printing fabrication," *J. Biomed. Mater. Res. B, Appl. Biomater.*, vol. 107, 5, pp. 1329–1351, 2019. doi: 10.1002/jbm.b.34226.
- [14] *Standard Terminology for Additive Manufacturing – General Principles – Terminology*. ASTM 52900, 2015.
- [15] L. Elomaa, S. Teixeira, R. Hakala, H. Korhonen, D. W. Grijpma, and J. V. Seppälä, "Preparation of poly (ϵ -caprolactone)-based tissue engineering scaffolds by stereolithography," *Acta Biomater.*, vol. 7, no. 11, pp. 3850–3856, 2011. doi: 10.1016/j.actbio.2011.06.039.
- [16] J. Bustillos, D. Montero-Zambrano, A. Loganathan, B. Boesl, and A. Agarwal, "Stereolithography-based 3D printed photosensitive polymer/boron nitride nanoscale composites," *Polym. Compos.*, vol. 40, no. 1, pp. 379–388, 2019. doi: 10.1002/pc.24662.
- [17] S. F. S. Shirazi et al., "A review on powder-based additive manufacturing for tissue engineering: Selective laser sintering and inkjet 3D printing," *Sci. Technol. Adv. Mater.*, vol. 16, no. 3, p. 033502, June 2015. doi: 10.1088/1468-6996/16/3/033502.
- [18] J. P. Kruth, G. Levy, F. Klocke, and T. H. C. Childs, "Consolidation phenomena in laser and powder-bed based layered manufacturing," *CIRP Ann.*, vol. 56, 2, pp. 730–759, 2007. doi: 10.1016/j.cirp.2007.10.004.
- [19] C. K. Chua, K. F. Leong, K. H. Tan, F. E. Wiria, and C. M. Cheah, "Development of tissue scaffolds using selective laser sintering of polyvinyl alcohol/hydroxyapatite biocomposite for craniofacial and joint defects," *J. Mater. Sci. Mater. Med.*, vol. 15, 10, pp. 1113–1121, 2004. doi: 10.1023/B:JMSM.0000046393.81449.a5.
- [20] W. Y. Zhou, S. H. Lee, M. Wang, W. L. Cheung, and W. Y. Ip, "Selective laser sintering of porous tissue engineering scaffolds from poly(L-lactide)/carbonated hydroxyapatite nanocomposite microspheres," *J. Mater. Sci. Mater. Med.*, vol. 19, no. 7, pp. 2535–2540, 2008. doi: 10.1007/s10856-007-3089-3.
- [21] B. Duan, M. Wang, W. Y. Zhou, W. L. Cheung, Z. Y. Li, and W. W. Lu, "Three-dimensional nanocomposite scaffolds fabricated via selective laser sintering for bone tissue engineering," *Acta Biomater.*, vol. 6, no. 12, pp. 4495–4505, 2010. doi: 10.1016/j.actbio.2010.06.024.
- [22] I. Zein, D. W. Hutmacher, K. C. Tan, and S. H. Teoh, "Fused deposition modeling of novel scaffold architectures for tissue engineering applications," *Biomaterials*, vol. 23, no. 4, pp. 1169–1185, 2002. doi: 10.1016/S0142-9612(01)00232-0.
- [23] A. Khojasteh, B. Behnia, F. S. Hosseini, M. M. Dehghan, P. Abbasnia, and F. M. Abbas, "The effect of PCL-TCP scaffold loaded with mesenchymal stem cells on vertical bone augmentation in dog mandible: A preliminary report," *J. Biomed. Mater. Res. B, Appl. Biomater.*, vol. 101, no. 5, pp. 848–854, 2013. doi: 10.1002/jbm.b.32889.
- [24] B. Rai et al., "Polycaprolactone-20% tricalcium phosphate scaffolds in combination with platelet-rich plasma for the treatment of critical-sized defects of the mandible: A pilot study," *J. Oral Maxillofac. Surg.*, vol. 65, no. 11, pp. 2195–2205, 2007. doi: 10.1016/j.joms.2006.11.026.
- [25] B. Rai, J. L. Lin, Z. X. H. Lim, R. E. Guldberg, D. W. Hutmacher, and S. M. Cool, "Differences between in vitro viability and differentiation and in vivo bone-forming efficacy of human mesenchymal stem cells cultured on PCL-TCP scaffolds," *Biomaterials*, vol. 31, no. 31, pp. 7960–7970, 2010. doi: 10.1016/j.biomaterials.2010.07.001.
- [26] N. Xu et al., "3D artificial bones for bone repair prepared by computed tomography-guided fused deposition modeling for bone repair," *ACS Appl. Mater. Interf.*, vol. 6, no. 17, pp. 14,952–14,963, 2014. doi: 10.1021/am502716t.
- [27] E. S. Bishop et al., "3D bioprinting technologies in tissue engineering and regenerative medicine: Current and future trends," *Genes Dis.*, vol. 4, no. 4, pp. 185–195, Dec. 2017. doi: 10.1016/j.gendis.2017.10.002.
- [28] A. V. Do, B. Khorsand, S. M. Geary, and A. K. Salem, "3D printing of scaffolds for tissue regeneration applications," *Adv. Healthc. Mater.*, vol. 4, no. 12, pp. 1742–1762, 2015. doi: 10.1002/adhm.201500168.
- [29] G. H. Wu and S. H. Hsu, "Review: Polymeric-based 3D printing for tissue engineering," *J. Med. Biol. Eng.*, vol. 35, no. 3, pp. 285–292, 2015. doi: 10.1007/s40846-015-0038-3.
- [30] J.-H. Lee and H.-W. Kim, "Emerging properties of hydrogels in tissue engineering," *J. Tissue Eng.*, vol. 9, p. 2041731418768285, Jan.–Dec. 2018. doi: 10.1177/2041731418768285.
- [31] P. Nezhad-Mokhtari, M. Ghorbani, L. Roshangar, and J. Soleimani Rad, "A review on the construction of hydrogel scaffolds by various chemically techniques for tissue engineering," *Eur. Polym. J.*, vol. 117, pp. 64–76, Aug. 2019. doi: 10.1016/j.eurpolymj.2019.05.004.
- [32] S. Vijayavenkataraman, W. C. Yan, W. F. Lu, C. H. Wang, and J. Y. H. Fuh, "3D bioprinting of tissues and organs for regenerative medicine," *Adv. Drug Deliv. Rev.*, vol. 132, pp. 296–332, July 2018. doi: 10.1016/j.addr.2018.07.004.
- [33] A. B. Dababneh and I. T. Ozbolat, "Bioprinting technology: A current state-of-the-art review," *J. Manuf. Sci. Eng.*, vol. 136, no. 6, p. 061016, 2014. doi: 10.1115/1.4028512.
- [34] J. M. Lee and W. Y. Yeong, "Design and printing strategies in 3D bioprinting of cell-hydrogels: A review," *Adv. Healthc. Mater.*, vol. 5, no. 22, pp. 2856–2865, 2016. doi: 10.1002/adhm.201600435.
- [35] S. V. Murphy and A. Atala, "3D bioprinting of tissues and organs," *Nat. Biotechnol.*, vol. 32, no. 8, pp. 773–785, 2014. doi: 10.1038/nbt.2958.
- [36] N. R. Schiele, D. T. Corr, Y. Huang, N. A. Raof, Y. Xie, and D. B. Chrisey, "Laser-based direct-write techniques for cell printing," *Biofabrication*, vol. 2, no. 3, p. 032001, 2010. doi: 10.1088/1758-5082/2/3/032001.
- [37] R. E. Saunders and B. Derby, "Inkjet printing biomaterials for tissue engineering: Bioprinting," *Int. Mater. Rev.*, vol. 59, no. 8, pp. 430–448, Nov. 2014. doi: 10.1179/1743280414Y.0000000040.
- [38] S. N. Jayasinghe, A. N. Qureshi, and P. A. M. Eagles, "Electrohydrodynamic jet processing: An advanced electric-field-driven jetting phenomenon for processing living cells," *Small*, vol. 2, no. 2, pp. 216–219, 2006. doi: 10.1002/smll.200500291.
- [39] S. Vijayavenkataraman, S. Zhang, S. Taharrah, G. Sriram, W. F. Lu, and J. Y. H. Fuh, "Electrohydrodynamic jet 3D printed nerve guide conduits (NGCs) for peripheral nerve injury repair," *Polymers*, vol. 10, no. 7, p. 753, 2018. doi: 10.3390/polym10070753. [Online]. Available: https://www.mdpi.com/2073-4360/10/7/753
- [40] S. Vijayavenkataraman, N. Vialli, J. Y. H. Fuh, and W. Feng Lu, "Conductive collagen/PPy-b-PCL hydrogel for bioprinting of neural tissue constructs," *Int. J. Bioprint.*, vol. 5, no. 2.1, pp. 31–43, 2019. doi: 10.18063/ijbv5i2.1.229.
- [41] S. Vijayavenkataraman, S. Kannan, T. Cao, J. Y. H. Fuh, G. Sriram, and W. F. Lu, "3D-printed PCL/PPy conductive scaffolds as three-dimensional porous nerve guide conduits (NGCs) for peripheral nerve injury repair," *Front. Bioeng. Biotechnol.*, vol. 7, p. 266, Oct. 2019. doi: 10.3389/fbioe.2019.00266.
- [42] U. Demirci and G. Montesano, "Single cell epitaxy by acoustic picolitre droplets," *Lab Chip*, vol. 7, no. 9, pp. 1139–1145, 2007. doi: 10.1039/b704965j.

- [43] W. L. Ng, J. M. Lee, W. Y. Yeong, and M. W. Naing, "Microvalve-based bioprinting—process, bio-inks and applications," *Biomater. Sci.*, vol. 5, no. 4, pp. 632–647, 2017. doi: 10.1039/C6BM00861E.
- [44] A. K. Miri et al., "Effective bioprinting resolution in tissue model fabrication," *Lab Chip*, vol. 19, no. 11, pp. 2019–2037, 2019. doi: 10.1039/C8LC01037D.
- [45] K. Hölzl, S. Lin, L. Tytgat, S. Van Vlierberghe, L. Gu, and A. Ovsianikov, "Bioink properties before, during and after 3D bioprinting," *Biofabrication*, vol. 8, no. 3, p. 032002, 2016. doi: 10.1088/1758-5090/8/3/032002.
- [46] E. L. Gill, X. Li, M. A. Birch, and Y. Y. S. Huang, "Multi-length scale bioprinting towards simulating microenvironmental cues," *Biores Manuf.*, vol. 1, no. 2, pp. 77–88, 2018. doi: 10.1007/s42242-018-0014-1.
- [47] N. Ashammakhi et al., "Bioinks and bioprinting technologies to make heterogeneous and biomimetic tissue constructs," *Mater. Today Bio*, vol. 1, p. 100,008, Jan. 2019. doi: 10.1016/j.mtbio.2019.100008.
- [48] E. Sodupe-Ortega, A. Sanz-Garcia, A. Pernia-Espinoza, and C. Escobedo-Lucea, "Accurate calibration in multi-material 3D bioprinting for tissue engineering," *Materials*, vol. 11, no. 8, p. 1402, Aug. 2018.
- [49] G. V. Shivashankar, M. Sheetz, and P. Matsudaira, "Mechanobiology," *Integr. Biol.*, vol. 7, no. 10, pp. 1091–1092, 2015. doi: 10.1039/C5IB90040A.
- [50] J. Eyckmans, T. Boudou, X. Yu, and C. S. Chen, "A hitchhiker's guide to mechanobiology," *Dev. Cell*, vol. 21, no. 1, pp. 35–47, 2011. doi: 10.1016/j.devcel.2011.06.015.
- [51] S. B. H. Timraz, R. Rezgui, S. M. Boularaoui, and J. C. M. Teo, "Stiffness of extracellular matrix components modulates the phenotype of human smooth muscle cells in vitro and allows for the control of properties of engineered tissues," *Procedia Eng.*, vol. 110, pp. 29–36, 2015. doi: 10.1016/j.proeng.2015.07.006. [Online]. Available: <https://www.sciencedirect.com/science/article/pii/S1877705815012473>
- [52] D. Kang et al., "Pre-set extrusion bioprinting for multiscale heterogeneous tissue structure fabrication," *Biofabrication*, vol. 10, no. 3, p. 035008, 2018. doi: 10.1088/1758-5090/aac70b.
- [53] J. Lee, K. E. Kim, S. Bang, I. Noh, and C. Lee, "A desktop multi-material 3D bio-printing system with open-source hardware and software," *Int. J. Precis. Eng. Manuf.*, vol. 18, no. 4, pp. 605–612, 2017. doi: 10.1007/s12541-017-0072-x.
- [54] J. Ma, Y. Wang, and J. Liu, "Bioprinting of 3D tissues/organs combined with microfluidics," *RSC Adv.*, vol. 8, no. 39, pp. 21,712–21,727, 2018. doi: 10.1039/C8RA03022G.
- [55] M. Rocca, A. Fragasso, W. Liu, M. A. Heinrich, and Y. S. Zhang, "Embedded multimaterial extrusion bioprinting," *SLAS Technol.*, vol. 23, no. 2, pp. 154–163, 2018. doi: 10.1177/2472630317742071.
- [56] T. J. Hinton et al., "Three-dimensional printing of complex biological structures by freeform reversible embedding of suspended hydrogels," *Sci. Adv.*, vol. 1, no. 9, p. e1500758, Oct. 2015. doi: 10.1126/sciadv.1500758.
- [57] W. Liu et al., "Rapid continuous multimaterial extrusion bioprinting," *Adv. Mater.*, vol. 29, no. 3, pp. 1–8, 2017. doi: 10.1002/adma.201770016.
- [58] M. Hu et al., "Cell immobilization in gelatin-hydroxyphenylpropionic acid hydrogel fibers," *Biomaterials*, vol. 30, no. 21, pp. 3523–3531, 2009. doi: 10.1016/j.biomaterials.2009.03.004.
- [59] Y. Zhang, Y. Yu, H. Chen, and I. T. Ozbolat, "Characterization of printable cellular microfluidic channels for tissue engineering," *Biofabrication*, vol. 5, no. 2, p. 025004, June 2013. doi: 10.1088/1758-5082/5/2/025004.
- [60] Q. Pi et al., "Digitally tunable microfluidic bioprinting of multilayered cannular tissues," *Adv. Mater.*, vol. 30, no. 43, p. 1,706,913, Oct. 2018. doi: 10.1002/adma.201706913.
- [61] S. Ghorbanian, M. A. Qasimeh, M. Akbari, A. Tamayol, and D. Juncker, "Microfluidic direct writer with integrated declogging mechanism for fabricating cell-laden hydrogel constructs," *Biomed. Microdevices*, vol. 16, no. 3, pp. 387–395, 2014. doi: 10.1007/s10544-014-9842-8.
- [62] L. Serex, A. Bertsch, and P. Renaud, "Microfluidics: A new layer of control for extrusion-based 3D printing," *Micromachines*, vol. 9, no. 2, p. 86, Feb. 2018. doi: 10.3390/mi9020086.
- [63] F. Yu and D. Choudhury, "Microfluidic bioprinting for organ-on-a-chip models," *Drug Discov. Today*, vol. 24, no. 6, pp. 1248–1257, June 2019. doi: 10.1016/j.drudis.2019.03.025.

